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Radiation use efficiency, chlorophyll fluorescence, and reflectance indices associated with ontogenic changes in water-limited *Chenopodium quinoa* leaves

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Abstract

Net photosynthetic rate, radiation use efficiency, chlorophyll (Chl) fluorescence, photochemical reflectance index (PRI), and leaf water potential were measured during a 25-d period of progressive water deficit in quinoa plants grown in a glasshouse in order to examine effects of water stress and ontogeny. All physiological parameters except F_v/F_m were sensitive to water stress. Ontogenic variations did not exist in F_v/F_m and leaf water potential, and were moderate to high in the other parameters. The complete recovery of photosynthetic parameters after re-irrigation was related with the stability in F_v/F_m . PRI showed significant correlation with predawn leaf water potential, F_m' , and midday F_v/F_m . Thus PRI and Chl fluorescence may help in assessing physiological changes in quinoa plants across different developmental stages and water status.

Additional key words: leaf water potential; net photosynthetic rate; PRI; quinoa; water stress.

Introduction

Non-invasive techniques such as Chl fluorescence and photochemical reflectance index (PRI) techniques have been developed in early 1980s and late 1980s, respectively, to detect changes in plant physiological status. In comparison with conventional ecophysiological methods such as measurements of net photosynthetic rate (P_N) or leaf water content, these methods are more informative with respect to localising photosynthetic apparatus disturbances (Méthy *et al.* 1991, 1996, Lichtenthaler 1996, Gamon *et al.* 1997). They also offer the potential for remote applications at scales larger than the leaf (Peñuelas and Filella 1998). Both techniques are related to the mechanisms of dissipation of excess energy in stressed plants that receive more photons than can be used for carbon assimilation. One of these mechanisms consists in the re-radiation of the excess energy as fluorescence emitted by Chl *a* of photosystem 2 (PS2) (Krause and Weis 1991). The other mechanism is heat dissipation, linked to the inter-conversion of the xantho-

phyll cycle pigments, and inducing changes in the leaf reflectance at 531 nm which are detected by PRI (Peñuelas *et al.* 1995, Gamon *et al.* 1997).

The effects of water stress on Chl fluorescence, PRI, and their relations with radiation use efficiency have been widely reported, but sometimes with scattered or contradictory result (Peñuelas *et al.* 1994, 1997). Ontogenic effects could explain part of these results, since photosynthetic processes underlying these eco-physiological techniques are sensitive not only to environmental stresses, but also to normal ontogenetic changes. Studies on herbaceous as well as perennial species show that changes in Chl fluorescence related to ontogeny can be larger than those induced by any stress factor or genotypic difference (Krebs *et al.* 1996, Šesták and Šiffel 1997, Synková *et al.* 1997, Araus *et al.* 1998, Gielen *et al.* 2000). The same kind of reasoning can be held for PRI where additional confounding influences (vegetation structure, calibration errors) can impact a relatively small

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Abbreviations: Chl – chlorophyll; DAE – days after emergence; F_m – maximal fluorescence in the dark-adapted state; F_m' – maximal fluorescence in the light-adapted state; F_0 – initial fluorescence; F_s – steady state value of fluorescence; F_v/F_m – photochemical efficiency of dark-adapted leaves; $\Delta F/F_m'$ – photochemical efficiency of photosystem 2; P_N – net photosynthetic rate; PPFD – photosynthetic photon fluence density; PRI – photochemical reflectance index; PRUE – photosynthetic radiation use efficiency; PS – photosystem; R_x – reflectance at x nm.

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signal (Gamon and Qiu 1999, Aparicio *et al.* 2000). It is thus essential to better understand the normal seasonal changes in Chl fluorescence and PRI to avoid interpreting them as indicative of stress damage when, in fact, they are normal part of the seasonal physiological cycle (Mohammed *et al.* 1995).

Chenopodium quinoa (Willd.) is a herbaceous, annual crop originated in the Andes, well-adapted to harsh environments characterised by drought, frost, soil salinity, and high solar visible and UV-B radiation (Tapia *et al.* 1979). It shows particular leaf properties such as epidermal vesicles (bladder cells), highly varying contents of leaf pigments, and high nitrogen concentration.

Materials and methods

Plants, growth conditions, and stress treatment: The experiment was carried out in a greenhouse at the Cefe-CNRS, Montpellier, France (43°36'N, 3°53'E). *C. quinoa* cv. Sajama was grown on a mixture of compost and sandy soil in 2 000 cm³ pots, at a density of one plant per pot and 36 pots per square meter. Pots were moved randomly every other day to avoid position effects. Sowing was on 27 January 2000, and emergence was on 31 January. Plants were irrigated with a complete nutritive solution (nitrogen 0.19 g m⁻³, phosphorus 0.1 g m⁻³, potassium 0.18 g m⁻³). Irrigation was applied daily at a dose equal to the average evapotranspiration calculated by weighing eight control pots daily. Stress treatment was applied progressively on half of the plants, reducing the irrigation in comparison to the control by 50 % from 46 to 52 d after emergence (DAE), by 66 % from 53 to 54 DAE, and by 75 % from 55 to 58 DAE. From 59 DAE on, full irrigation was again applied to all the plants to study stress recovery in re-irrigated plants. The experiment was over at 69 DAE.

Physiological measurements: Chl fluorescence, PRI, and photosynthesis parameters were measured on the adaxial surface of four top-canopy, fully expanded leaves randomly selected on four plants in each water treatment. Leaf water potential was measured at predawn and at noon, on similar leaves with three replicates in each water treatment. Due to technical constraints, data collection for the various parameters could not be made on the same day, but was made over two consecutive days during the stress period, and three to six days during the recovering period.

Chl fluorescence was measured with a PAM-2000 fluorometer (H. Walz, Germany) using the saturation pulse method (Schreiber *et al.* 1986, Schreiber and Bilger 1987). Initial Chl fluorescence, F_0 , was induced by applying a weak modulated measuring radiation (0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 655 nm) at a frequency of 600 Hz on the adaxial surface of dark-adapted leaves. Using red excitation radiation allows to minimise the absorption by the photosynthetic accessory pigments of the pigment antenna

These leaf properties present large genotypic and ontogenetic variations, but their functional role remains largely unknown (Jacobsen and Mujica Sánchez 1999). Chl fluorescence and PRI techniques would then help the rapid and non-invasive assessment of functional heterogeneity within and among quinoa populations. Prior to this, information is needed on the performance of these techniques in assessing physiological and ontogenic changes in the particular case of *C. quinoa*. Thus, the aim of this study was to analyse the variations in Chl fluorescence, PRI, P_N , and leaf water potential in quinoa plants during a 25-d period of progressive water deficit and recovering at the pre-flowering and anthesis stages.

(Chl *a*, Chl *b*, carotenoids) and reaches deeper cell and chloroplast layers inside the leaf than shorter wavelengths (Rinderle and Lichtenthaler 1988). Leaf clips (PEALC, Hansatech, UK) were used for the dark adaptation (30 min) of the samples. The F_0 level is Chl fluorescence when all reaction centres are open, and the rate of PS2 photochemistry is not limited. It may increase if the PS2 reaction centres are damaged, or if the transfer of excitation energy from the antenna to the reaction centres is impeded. Maximum Chl fluorescence in the dark-adapted stage (F_m) was determined using an 800 ms pulse of high irradiance "white light" (12 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, <710 nm). The steady-state value of fluorescence F_s was measured, and a second pulse of high irradiance white light was used to determine maximum Chl fluorescence in the light-adapted phase (F_m'). The photochemical efficiencies of PS2 in the dark (maximum PS2 photochemical efficiency) and in the light (effective quantum yield of PS2) were calculated according to Genty *et al.* (1989):

$$F_v/F_m = (F_m - F_0)/F_m$$

$$\Delta F/F_m' = (F_m' - F_s)/F_m'$$

F_v/F_m was measured predawn and at midday. $\Delta F/F_m'$ was measured during the day at photosynthetic photon flux densities (PPFD) higher than 1 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

PRI was measured using a two-channel fiber optic radiometer (SKR 116/SKP 120, Sky Instrument, UK) equipped with interference optic filters transmitting the 530 and 570 nm wavelengths with a bandwidth of 10±2 nm. Reflectance spectra were calculated by dividing the spectral radiance of the adaxial surface of the leaf by the radiance of a 99 % reflective white standard (Spectralon, Labsphere, USA). PRI was formulated following Peñuelas *et al.* (1995):

$$\text{PRI} = (R_{530} - R_{570})/(R_{530} + R_{570})$$

where R_{530} indicates reflectance at 530 nm (due to the availability of interference filters, this waveband was used in place of 531 nm, the waveband of the "xanthophyll signal") and R_{570} indicates reflectance at 570 nm (a reference waveband). By referencing R_{530} against R_{570} ,



this index partly reduces the effect of other factors besides the xanthophyll cycle, such as chloroplast movements, that can affect reflectance in this spectral region (Gamon *et al.* 1997). PRI was measured during the day at PPF higher than $1\ 600\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$.

P_N was measured with a portable photosynthesis system (LI-6400, Li-Cor, USA). Air temperature and humidity in the cuvette varied between 18 and 24 °C, and 16 and 50 %, respectively, while input air CO_2 concentration was between 325 and $392\ \mu\text{mol}\ \text{mol}^{-1}$. Irradiance-response curves were registered within 40 min in the range $0\text{--}1\ 000\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ PPF, and within 15 min in the range $1\ 000\text{--}1\ 870\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ PPF. Values corresponding to PPF between $1\ 775$ and $1\ 870\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ were used to estimate the photon-saturated P_N [$\mu\text{mol}(\text{CO}_2)\ \text{m}^{-2}\ \text{s}^{-1}$] and the instantaneous photosynthetic

Results

Kinetics: In control plants, predawn leaf water potential varied little during the experiment (mean value of $-0.45\ \text{MPa} \pm 11\%$, Fig. 1A), and minimum leaf water potential remained between -0.6 and $-1.07\ \text{MPa}$ (Fig. 1B). In stressed plants, the progressive reduction of irrigation induced a fall in predawn leaf water potential statistically significant after 12 d, and reaching $-0.97\ \text{MPa}$ after 14 d. The effect of water stress was more rapid on the minimum leaf water potential, statistically lower than the control since the 10th d of deficit. Minimum leaf water potential reached $-1.38\ \text{MPa}$ after 12 d of water deficit. Then, two days of re-irrigation were enough for the complete recovering of water status in formerly stressed plants.

PRUE in control plants (Fig. 1C) was stable at the beginning of the experiment [mean value of the first 3 observations = $10.7\ \text{mmol}(\text{CO}_2)\ \text{mol}^{-1}(\text{PPFD}) \pm 5\%$], then it decreased by 30 % by the end of the period (significant difference, $p < 0.001$). In stressed plants, PRUE remained similar to that of control plants in a first period (53 DAE), but after 13 d of irrigation deficit water stress induced a significant drop in P_N (-84% relative to the control). In these stressed plants, re-irrigation allowed for the complete recovering of PRUE, which even appeared statistically higher than in control plants by the end of the experiment (69 DAE). P_N showed essentially the same variations as PRUE (values not shown), since the range of PPF was narrow, and was highly correlated with stomatal conductance ($r = 0.92$, $n = 28$). P_N continued to increase up to PPF of $1\ 870\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$, indicating that photoinhibition did not occur at the irradiances used in this experiment.

PRI in control plants increased regularly with time, from -0.07 to -0.02 between 46 and 69 DAE (Fig. 1D). The same trend was observed on stressed plants, yet with an increase higher than in the control plants by the end of

radiation use efficiency, PRUE [$\text{mmol}(\text{CO}_2)\ \text{mol}^{-1}(\text{PPFD})$].

As an index of plant water stress, leaf water potential was measured predawn, Ψ_p [MPa] and near midday, Ψ_m [MPa] using a Scholander pressure chamber (PMS Instrument, USA).

Statistics: Means of control and stress treatments were compared using Student *t*-tests. Relations between PRI and the other physiological variables were analysed using Pearson correlation coefficients and linear regressions calculated from the four means of the control treatment corresponding to the four periods when measurements of the various parameters were coincident (on Fig. 2 these four periods are indicated in chronological order by *a*, *b*, *c*, and *d*).

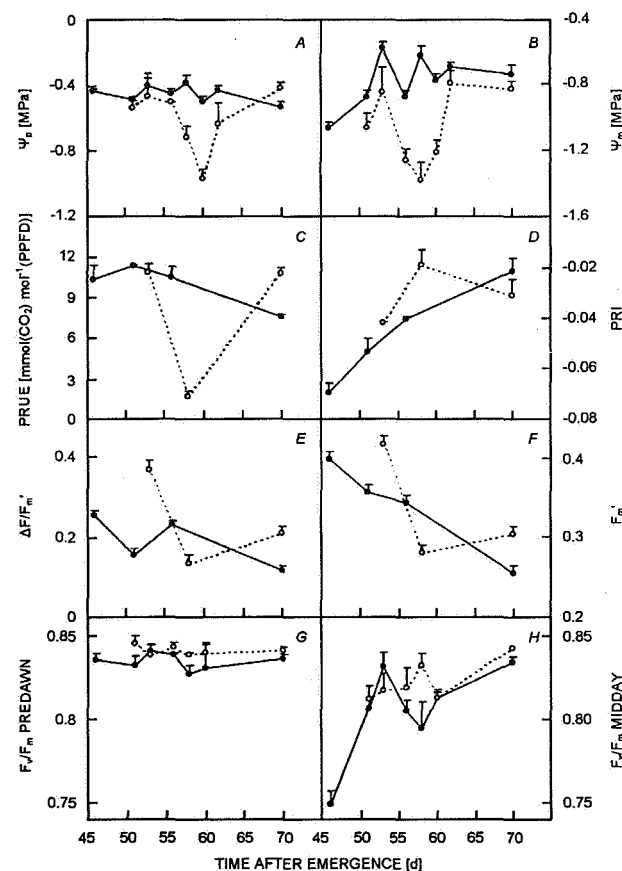


Fig. 1. Mean values of predawn (A) and midday (B) leaf water potential, photosynthetic radiation-use efficiency (C), photochemical reflectance index (D), $\Delta F/F_m'$ (E), F_m' (F), predawn (G) and midday (H) F_v/F_m in control (closed symbols) and water stressed (open symbols) top-canopy, fully expanded quinoa leaves during plant ontogeny (vertical bars show one standard error).

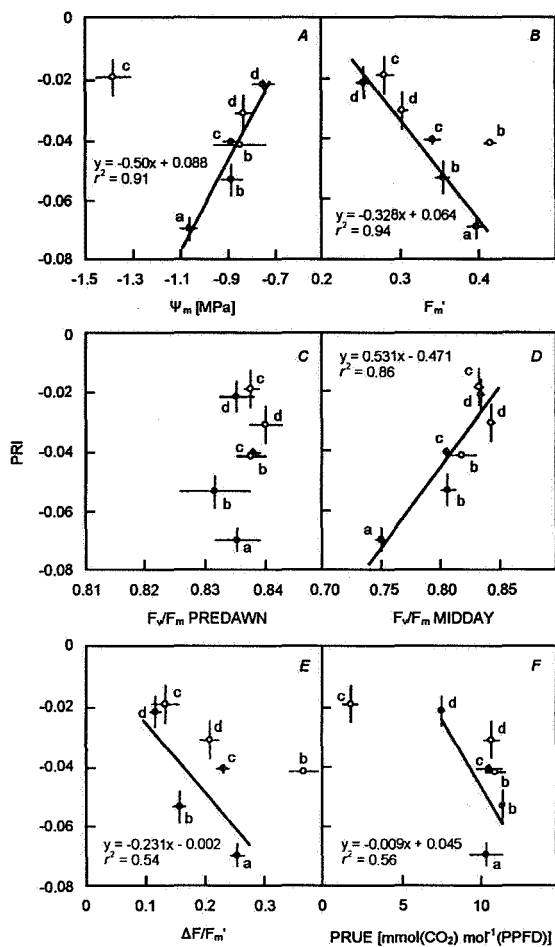


Fig. 2. Relations between photochemical reflectance index (PRI) and midday leaf water potential (A), F_m' (B), predawn F_v/F_m (C), midday F_v/F_m (D), $\Delta F/F_m'$ (E), and photosynthetic radiation use efficiency, PRUE (F) in control (closed symbols) and water stressed (open symbols) top-canopy, fully expanded quinoa leaves during plant ontogeny (vertical bars show one standard error, letters show successive time periods). Regressions were calculated for the control treatment ($n = 4$).

Discussion

Effects of water deficit versus ontogenic effects: Although progressive, the water deficit had a pronounced effect on leaf water potential, P_N , and radiation-use efficiency. This stress effect appeared more rapidly in minimum leaf water potential than in the other physiological parameters. But in all cases, the effect of water deficit was reversible, and few days of re-irrigation allowed for the complete recovering of the leaf water status, P_N , and radiation-use efficiency. Rasmussen (1997) also observed this fast recovery after a drought stress period. It is consistent with the stability in predawn F_v/F_m , indicative of the maintenance of a high photo-chemical efficiency in the dark phase despite the water deficit. The level of water stress reached in this experiment (-1.4 MPa) was comparable to that observed at the pre-flowering and

the stress period (58 DAE). No statistical difference was shown between treatments.

The Chl fluorescence parameters $\Delta F/F_m'$ and F_m' (Fig. 1E,F) decreased with time in control plants, while in stressed ones their evolution was more complex: an increase at the beginning of the period of water deficit was followed by an important decrease by the end of this period. Then a new increase in the re-irrigation period followed, with deviations from the control statistically significant in each occasion.

Predawn values of F_v/F_m remained stable during the experiment, without significant differences between the treatments (Fig. 1G). Midday values measured in control plants showed a large increase at the beginning, then remained relatively stable around $0.81 \pm 3\%$ (Fig. 1H). No difference appeared with stressed plants during or after the water deficit period.

Relationships between PRI and other physiological variables: PRI was positively correlated with minimum leaf water potential in control plants ($r = 0.95$, $p = 0.067$, Fig. 2A). Stressed plants showed the same relation as long as water stress was mild or in the recovering period (Fig. 2A, points b and d). PRI was negatively correlated with F_m' in control plants ($r = -0.97$, $p = 0.031$, Fig. 2B). The same linear relation applied to stressed plants, except for the data of 53 DAE (Fig. 2B, point b) probably because of the complex variations in F_m' in stressed plants. A positive correlation was found between PRI and midday F_v/F_m in control plants ($r = 0.93$, $p = 0.067$, Fig. 2D). Stressed plants followed the same relation whatever the treatment phase (stress or recovering). PRI did not show any significant relation with predawn leaf water potential, PRUE, $\Delta F/F_m'$, or predawn F_v/F_m . However, Fig. 2E,F suggests an appreciable increase in PRI in relation to small decreases in $\Delta F/F_m'$ or PRUE, as those observed in control plants.

flowering stages in quinoa plants grown in pots (Jensen *et al.* 2000). But it appeared much less severe than that observed on quinoa plants subjected to post-flowering drought in the Bolivian Altiplano (Vacher 1998). The difference could be due to the greater intensity of water deficit combined with higher evaporative demand of the atmosphere in the conditions of Altiplano. But it could also be explained by the difference in the development stage (maturity in the cited study, pre-flowering and anthesis in our case), as leaf water potential tends to decline rapidly in senescing plants (Karamanos and Papatheohari 1999, Mastroirilli *et al.* 1999). In our experiment limited to the pre-flowering and anthesis phase, water status parameters in control plants remained stable and did not show ontogenic variations, while P_N and PRUE, stable in

the first two weeks of the experiment, declined significantly by the end of the period. This trend is in agreement with the observations of Jensen *et al.* (2000) on changes in P_N during the flowering and grain filling phases. It corresponds to the decrease in radiation-use efficiency observed in other species during late ontogeny (Hodáňová 1981, Scartazza *et al.* 1998, Reynolds *et al.* 2000) and coincides with decreases in F_m' and $\Delta F/F_m'$ indicative of lower photo-chemical efficiency in the light phase by the end of the period.

On the other hand, ontogenic variations were clear and consistent in PRI, F_m' , and $\Delta F/F_m'$. During the four weeks of experiment, and in the absence of water stress (control plants), PRI increased by 70 %, while F_m' and $\Delta F/F_m'$ dropped by 36 and 55 %, respectively. On the opposite, the impact of the water deficit on PRI and the Chl fluorescence parameters was nil (case of F_v/F_m) or without any clear pattern (case of PRI, $\Delta F/F_m'$, and F_m'). Regarding PRI, by the end of the water deficit period the stressed plants showed values not only higher by 50 % but also much more variable (CV = 56 %) than the control. Due to this variability, PRI in stressed plants did not appear statistically larger than in the control. Such variability in PRI could reflect functional heterogeneity in quinoa plants under stress. In the particular case of $\Delta F/F_m'$ and F_m' , the rapid variations observed in stressed plants may reveal changes in leaf optical properties, in relation to the alteration of the epidermal vesicles under the effect of water stress as observed by Adams *et al.* (1998) in *Mesembryanthemum crystallinum*.

Chl fluorescence and PRI as tools for drought stress assessment in *C. quinoa*: Our results showed consistent correlation, at leaf level, in dark-adapted state, between PRI and maximal photochemical efficiency of PS2 (F_v/F_m). Application of water stress caused an increase in PRI in the light-adapted state, with negative correlation with PRUE and $\Delta F/F_m'$. Similar results, unobservable with nutrient stress, were presented by Peñuelas *et al.* (1994) with water-stressed sunflower plants. However,

most of the correlation between PRI and F_v/F_m relies on ontogenetic changes during the three weeks of experimentation. A highly significant relationship also occurred between PRI and F_m' . This result was not expected since F_m' is not normalised against any other Chl fluorescence parameter and should present high intrinsic variability. Santos *et al.* (1998) found F_m' highly correlated with the rate of electron transport through PS2 in infected cacao genotypes. Although F_m' is often neglected as a parameter, these results suggest its interest in assessing the physiological status of plants across different genotypes, developmental stages, and environmental conditions.

Among other objectives, the present work aimed to test, by a case study, the use of Chl fluorescence and PRI signatures as tools for detection of water stress tolerance and stress-induced injuries. The above results confirm that Chl fluorescence induction, using the saturation pulse method associated to the pulse-amplitude-modulation technique, is a powerful tool, perfected for studying stress physiology and related photosynthetic processes at leaf level. However, non-imaging fiber-optic bundles average information over one to several square centimetres of leaf surface. Moreover, notwithstanding promising results (Günther *et al.* 1994, Cerovic *et al.* 1996), it is known that the Chl fluorescence standard techniques are still not always sufficiently suitable for remote sensing. Imaging fluorometers, laser-induced or flash-light induced fluorescence imaging systems (Lang *et al.* 1996, Sowinska *et al.* 1999, Lootens and Vandecasteele 2000) are particularly well-suited for the study of targets displaying heterogeneities in photosynthetic activities. Taking into account the particular leaf properties of *C. quinoa*, this patchiness needs to be further investigated. PRI is suited for remote study of photosynthetic function, and promising results were obtained at the leaf and canopy levels (Peñuelas *et al.* 1995, Aparicio *et al.* 2000, Nichol *et al.* 2000). However, emphasis was laid on canopy structure induced problems (Gamon and Qiu 1999, Méthy 2000). These behaviours of PRI need also to be further investigated.

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